SEQUENCE LISTING

<110> Saxena, Shailendra K.

<120> RIBONUCLEASES AND METHODS OF MAKING THEM

<130> 5016 US

<160> 74

<170> PatentIn version 3.1

<210> 1
<211> 114
<212> PRT
<213> Artificial

<220>
<223> Recombinantly produced 2325p4 protein occurring naturally in rana pipiens eggs and embryos.

<400> 1

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Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His Ile
1               5                  10                  15

Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn Asp
            20                  25                  30

Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe Ile
        35                  40                  45

His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr Gly
    50                  55                  60

Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Thr Leu Thr Thr Cys Lys
65                  70                  75                  80

Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe Ile
                85                  90                  95

Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr Gly
            100                 105                 110

Lys Cys
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<210> 2
<211> 342
<212> DNA
<213> Artificial

<220>
<223> 2325p4 DNA occurring naturally in rana pipiens eggs and embryos.

<400> 2
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gttgctgact tcaactgcaa ccgtactatg aacgacccgg cttacactcc ggacggtcag 120

tgcaaaccga tcaacacttt catccattct actactggtc cggttaaaga aatctgccgt 180

cgtgctactg gtcgtgttaa caaatcttct actcagcagt tcactctgac tacttgcaaa 240

aacccgatcc gttgcaaata ctctcagtct aacactacta acttcatctg catcacttgc 300

cgtgacaact acccggttca tttcgttaaa actggtaaat gc 342

<210> 3
<211> 56
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:3 Contains XbaI restriction site.

<400> 3
taattttgtt taactttaag aaggagatat accatgaaac cgaaagaaga ccgtga 56

<210> 4
<211> 63
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:4 Complementary to SEQ ID NO:3

<400> 4
ttcccattca cggtcttctt tcggtttcat ggtatatctc cttcttaaag ttaaacaaaa 60

tta 63

<210> 5
<211> 57
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:5

<400> 5
atgggaaaaa ttcaaaacta aacatatcac ttctcagtct gttgctgact tcaactg 57

<210> 6
<211> 57
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:6 Complementary to SEQ ID NO:5

<400> 6
acggttgcag ttgaagtcag caacagactg agaagtgata tgtttagttt tgaattt 57

<210> 7
<211> 60
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:7

<400> 7
caaccgtact atgaacgacc cggcttacac tccggacggt cagtgcaaac cgatcaacac 60

<210> 8
<211> 60
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:8 Complementary to SEQ ID NO:7

<400> 8
gatgaaagtg ttgatcggtt tgcactgacc gtccggagtg taagccgggt cgttcatagt 60

<210> 9
<211> 52
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:9

<400> 9
tttcatccat tctactactg gtccggttaa agaaatctgc cgtcgtgcta ct 52

<210> 10
<211> 52
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:10 Complementary to SEQ ID NO:9

<400> 10
cacgaccagt agcacgacgg cagatttctt taaccggacc agtagtagaa tg 52

<210> 11
<211> 54
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:11

<400> 11
ggtcgtgtta acaaatcttc tactcagcag ttcactctga ctacttgcaa aaac 54

<210> 12
<211> 54

<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:12 Complementary to SEQ ID NO:11

<400> 12
ggatcgggtt tttgcaagta gtcagagtga actgctgagt agaagatttg ttaa 54

<210> 13
<211> 57
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:13

<400> 13
ccgatccgtt gcaaatactc tcagtctaac actactaact tcatctgcat cacttgc 57

<210> 14
<211> 57
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:14 Complementary to SEQ ID NO:13

<400> 14
tgtcacggca agtgatgcag atgaagttag tagtgttaga ctgagagtat ttgcaac 57

<210> 15
<211> 60
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:15

<400> 15
cgtgacaact acccggttca tttcgttaaa actggtaaat gctagtaggg atccgcgcgg 60

<210> 16
<211> 53
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:16 Complementary to SEQ ID NO:15

<400> 16
ccgcgcggat ccctactagc atttaccagt tttaacgaaa tgaaccgggt agt 53

<210> 17
<211> 114
<212> PRT
<213> Artificial

<220>
<223> Recombinantly produced 2325p6 protein occurring naturally in rana pipiens eggs and embryos.

<400> 17

Lys Pro Lys Glu Asp Lys Glu Trp Glu Lys Phe Lys Val Lys His Ile
1 5 10 15

Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Thr Ser Thr Met Asn Asn
20 25 30

Pro Asp Phe Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe Ile
35 40 45

His Ser Asn Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Ser Gly
50 55 60

Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Pro Leu Thr Thr Cys Lys
65 70 75 80

Asn Pro Lys Arg Cys Lys Tyr Ser Gln Ser Asn Glu Thr Asn Tyr Ile
85 90 95

Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Ile Gly
100 105 110

Lys Cys

<210> 18
<211> 342
<212> DNA
<213> Artificial

<220>
<223> 2325p6 DNA occurring naturally in rana pipiens eggs and embryos.

<400> 18
aaaccgaaag aagacaaaga atgggaaaaa ttcaaagtta aacatatcac ttctcagtct 60

gttgctgact tcaactgcac ttctactatg aacaacccgg acttcactcc ggacggtcag 120

tgcaaaccga tcaacacttt catccattct aacactggtc cggttaaaga aatctgccgt 180

cgtgcttctg gtcgtgttaa caaatcttct actcagcagt tcccgctgac tacttgcaaa 240

aacccgaaac gttgcaaata ctctcagtct aacgaaacta actacatctg catcacttgc 300

cgtgacaact acccggttca tttcgttaaa atcggtaaat gc 342

<210> 19
<211> 56
<212> DNA

<213> Artificial

<220>
<223> SEQ ID NO:19

<400> 19
taattttgtt taactttaag aaggagatat accatgaaac cgaaagaaga caaaga 56

<210> 20
<211> 63
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:20 Complementary to SEQ ID NO:19

<400> 20
ttcccattct ttgtcttctt tcggtttcat ggtatatctc cttcttaaag ttaaacaaaa 60

tta 63

<210> 21
<211> 57
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:21

<400> 21
atgggaaaaa ttcaaagtta aacatatcac ttctcagtct gttgctgact tcaactg 57

<210> 22
<211> 57
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:22 Complementary to SEQ ID NO:21

<400> 22
agaagtgcag ttgaagtcag caacagactg agaagtgata tgtttaactt tgaattt 57

<210> 23
<211> 60
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:23

<400> 23
cacttctact atgaacaacc cggacttcac tccggacggt cagtgcaaac cgatcaacac 60

<210> 24
<211> 60
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:24 Complementary to SEQ ID NO:23

<400> 24
gatgaaagtg ttgatcggtt tgcactgacc gtccggagtg aagtccgggt tgttcatagt 60

<210> 25
<211> 52
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:25

<400> 25
tttcatccat tctaacactg gtccggttaa agaaatctgc cgtcgtgctt ct 52

<210> 26
<211> 52
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:26 Complementary to SEQ ID NO:25

<400> 26
cacgaccaga agcacgacgg cagatttctt taaccggacc agtgttagaa tg 52

<210> 27
<211> 54
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:27

<400> 27
ggtcgtgtta acaaatcttc tactcagcag ttcccgctga ctacttgcaa aaac 54

<210> 28
<211> 54
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:28 Complement to SEQ ID NO:27

<400> 28
gtttcgggtt tttgcaagta gtcagcggga actgctgagt agaagatttg ttaa 54

<210> 29
<211> 57
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:29

<400> 29
ccgaaacgtt gcaaatactc tcagtctaac gaaactaact acatctgcat cacttgc 57

<210> 30
<211> 57
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:30 Complement to SEQ ID NO:29

<400> 30
tgtcacggca agtgatgcag atgtagttag tttcgttaga ctgagagtat ttgcaac 57

<210> 31
<211> 60
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:31 Contains stop codon and BamHI site

<400> 31
cgtgacaact acccggttca tttcgttaaa atcggtaaat gctagtaggg atccgcgcgg 60

<210> 32
<211> 53
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:32 Complementary to SEQ ID NO:31

<400> 32
ccgcgcggat ccctactagc atttaccgat tttaacgaaa tgaaccgggt agt 53

<210> 33
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> SEQ ID NO:33 pET-11d Forward primer contains XbaI site

<400> 33
caattcccct ctagaaataa ttttgtttaa ctttaagaag gag 43

<210> 34
<211> 114
<212> PRT
<213> Artificial

<220>
<223> Recombinantly produced 2728 protein occurring naturally in rana p
ipiens eggs and embryos.

<400> 34

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Lys Pro Lys Glu Asp Lys Glu Trp Val Lys Phe Lys Ala Lys His Ile
1               5                  10                  15

Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Lys Thr Met Asn Asp
            20                  25                  30

Pro Asp Phe Thr Pro Asp Gly Gln Cys Lys Pro Val Asn Thr Phe Ile
        35                  40                  45

His Ser Asn Thr Gly Pro Val Lys Asp Ile Cys Arg Arg Ala Ser Gly
    50                  55                  60

Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Pro Leu Thr Thr Cys Asn
65                  70                  75                  80

Lys Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe Ile
                85                  90                  95

Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Ile Gly
            100                 105                 110

Lys Cys
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<210> 35
<211> 342
<212> DNA
<213> Artificial

<220>
<223> 2728 DNA occurring naturally in rana pipiens eggs and embryos.

<400> 35
aaaccgaaag aagacaaaga atgggttaaa ttcaaagcta aacatatcac ttctcagtct 60

gttgctgact tcaactgcaa caaaactatg aacgacccgg acttcactcc ggacggtcag 120

tgcaaaccgg ttaacacttt catccattct aacactggtc cggttaaaga catctgccgt 180

cgtgcttctg gtcgtgttaa caaatcttct actcagcagt tcccgctgac tacttgcaac 240

aaaccgatcc gttgcaaata ctctcagtct aacactacta acttcatctg catcacttgc 300

cgtgacaact acccggttca tttcgttaaa atcggtaaat gc 342

<210> 36
<211> 56
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:36

<400> 36
aattttgttt aactttaaga aggagatata catatgaaac cgaaagaaga caaaga 56

<210> 37
<211> 56
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:37 Complement to SEQ ID NO:36

<400> 37
aacccattct ttgtcttctt tcggtttcat atgtatatct ccttcttaaa gttaaa 56

<210> 38
<211> 56
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:38

<400> 38
atgggttaaa ttcaaagcta aacatatcac ttctcagtct gttgctgact tcaact 56

<210> 39
<211> 56
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:39 Complement to SEQ ID NO:38

<400> 39
ttgttgcagt tgaagtcagc aacagactga gaagtgatat gtttagcttt gaattt 56

<210> 40
<211> 59
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:40

<400> 40
gcaacaaaac tatgaacgac ccggacttca ctccggacgg tcagtgcaaa ccggttaac 59

<210> 41
<211> 59
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:41 Complementary to SEQ ID NO:40

<400> 41
tgaaagtgtt aaccggtttg cactgaccgt ccggagtgaa gtccgggtcg ttcatagtt 59

<210> 42
<211> 54
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:42

<400> 42
actttcatcc attctaacac tggtccggtt aaagacatct gccgtcgtgc ttct 54

<210> 43
<211> 54
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:43 Complementary to SEQ ID NO:42

<400> 43
cacgaccaga agcacgacgg cagatgtctt taaccggacc agtgttagaa tgga 54

<210> 44
<211> 54
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:44

<400> 44
ggtcgtgtta acaaatcttc tactcagcag ttcccgctga ctacttgcaa caaa 54

<210> 45
<211> 54
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:45 Complementary to SEQ ID NO:44

<400> 45
ggatcggttt gttgcaagta gtcagcggga actgctgagt agaagatttg ttaa 54

<210> 46
<211> 57
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:46

<400> 46
ccgatccgtt gcaaatactc tcagtctaac actactaact tcatctgcat cacttgc 57

<210> 47
<211> 57

<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:47 Complementary to SEQ ID NO:46

<400> 47
tgtcacggca agtgatgcag atgaagttag tagtgttaga ctgagagtat ttgcaac 57

<210> 48
<211> 54
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:48

<400> 48
cgtgacaact acccggttca tttcgttaaa atcggtaaat gctagtaggg atcc 54

<210> 49
<211> 53
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:49 Complementary to SEQ ID NO:48

<400> 49
ccgcgcggat ccctactagc atttaccgat tttaacgaaa tgaaccgggt agt 53

<210> 50
<211> 42
<212> DNA
<213> Artificial

<220>
<223> pET-22b Forward primer contains XbaI site

<400> 50
gcccagccgg cgatggccaa accgaaagaa gaccgtgaat gg 42

<210> 51
<211> 114
<212> PRT
<213> Artificial

<220>
<223> Recombinantly produced 2325p4a protein occurring naturally in ran
a pipiens eggs and embryos.

<400> 51

Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His Ile
1 5 10 15

Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn Asp
20 25 30

Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Val Asn Thr Phe Ile
35 40 45

His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr Gly
50 55 60

Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Thr Leu Thr Thr Cys Lys
65 70 75 80

Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe Ile
85 90 95

Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr Gly
100 105 110

Lys Cys

<210> 52
<211> 342
<212> DNA
<213> Artificial

<220>
<223> 2325p4a DNA occurring naturally in rana pipiens eggs and embryos.

<400> 52
aaaccgaaag aagaccgtga atgggaaaaa ttcaaaacta aacatatcac ttctcagtct 60

gttgctgact tcaactgcaa ccgtactatg aacgacccgg cttacactcc ggacggtcag 120

tgcaaaccgg ttaacacttt catccattct actactggtc cggttaaaga aatctgccgt 180

cgtgctactg gtcgtgttaa caaatcttct actcagcagt tcactctgac tacttgcaaa 240

aacccgatcc gttgcaaata ctctcagtct aacactacta acttcatctg catcacttgc 300

cgtgacaact acccggttca tttcgttaaa actggtaaat gc 342

<210> 53
<211> 39
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:53 for substituting valine for isoleucine at position 4
4.

<400> 53
gacggtcagt gcaaaccggt taacactttc atccattct 39

<210> 54
<211> 39

<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:54 Complementary to SEQ ID NO:53

<400> 54
agaatggatg aaagtgttaa ccggtttgca ctgaccgtc 39

<210> 55
<211> 114
<212> PRT
<213> Artificial

<220>
<223> "Cysteinized" protein.

<400> 55

Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His Ile
1 5 10 15

Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn Asp
20 25 30

Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe Ile
35 40 45

His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr Gly
50 55 60

Arg Val Asn Lys Ser Ser Cys Gln Gln Phe Thr Leu Thr Thr Cys Lys
65 70 75 80

Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe Ile
85 90 95

Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr Gly
100 105 110

Lys Cys

<210> 56
<211> 342
<212> DNA
<213> Artificial

<220>
<223> DNA of "cysteinized" protein

<400> 56
aaaccgaaag aagaccgtga atgggaaaaa ttcaaaacta aacatatcac ttctcagtct 60

gttgctgact tcaactgcaa ccgtactatg aacgacccgg cttacactcc ggacggtcag 120

tgcaaaccga tcaacacttt catccattct actactggtc cggttaaaga aatctgccgt 180

cgtgctactg gtcgtgttaa caaatcttct tgccagcagt tcactctgac tacttgcaaa 240

aacccgatcc gttgcaaata ctctcagtct aacactacta acttcatctg catcacttgc 300

cgtgacaact acccggttca tttcgttaaa actggtaaat gc 342

<210> 57
<211> 39
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:57 Forward primer for substituting cysteine for threonine at position 71.

<400> 57
gttaacaaat cttcttgcca gcagttcact ctgactact 39

<210> 58
<211> 39
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:58 Reverse primer Complementary to SEQ ID NO:57

<400> 58
cagagtgaac tgctggcaag aagatttgtt aacacgacc 39

<210> 59
<211> 115
<212> PRT
<213> Artificial

<220>
<223> Recombinantly produced 2325p4 protein with methionine in -1 position.

<400> 59

Met Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His
1 5 10 15

Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn
20 25 30

Asp Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe
35 40 45

Ile His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr
50 55 60

Gly Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Thr Leu Thr Thr Cys
65 70 75 80

Lys Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe
85 90 95

Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr
100 105 110

Gly Lys Cys
115

<210> 60
<211> 115
<212> PRT
<213> Artificial

<220>
<223> Recombinantly produced 2325p6 protein with methionine in -1 position.

<400> 60

Met Lys Pro Lys Glu Asp Lys Glu Trp Glu Lys Phe Lys Val Lys His
1 5 10 15

Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Thr Ser Thr Met Asn
20 25 30

Asn Pro Asp Phe Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe
35 40 45

Ile His Ser Asn Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Ser
50 55 60

Gly Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Pro Leu Thr Thr Cys
65 70 75 80

Lys Asn Pro Lys Arg Cys Lys Tyr Ser Gln Ser Asn Glu Thr Asn Tyr
85 90 95

Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Ile
100 105 110

Gly Lys Cys
115

<210> 61
<211> 115
<212> PRT
<213> Artificial

<220>
<223> Recombinantly produced 2728 protein with methionine in -1 positio
n.

<400> 61

Met Lys Pro Lys Glu Asp Lys Glu Trp Val Lys Phe Lys Ala Lys His
1 5 10 15

Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Lys Thr Met Asn
20 25 30

Asp Pro Asp Phe Thr Pro Asp Gly Gln Cys Lys Pro Val Asn Thr Phe
35 40 45

Ile His Ser Asn Thr Gly Pro Val Lys Asp Ile Cys Arg Arg Ala Ser
50 55 60

Gly Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Pro Leu Thr Thr Cys
65 70 75 80

Asn Lys Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe
85 90 95

Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Ile
100 105 110

Gly Lys Cys
115

<210> 62
<211> 2
<212> PRT
<213> Artificial

<220>
<223> 2 residues of a pelB leader sequence

<400> 62

Met Ala
1

<210> 63
<211> 116
<212> PRT
<213> Artificial

<220>
<223> Recombinantly produced 2325p4 protein with pelB leader sequence t
hat is 2 amino acid residues long.

<400> 63

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Met Ala Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys
1               5                  10                  15

His Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met
            20                  25                  30

Asn Asp Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr
        35                  40                  45

Phe Ile His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala
    50                  55                  60

Thr Gly Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Thr Leu Thr Thr
65                  70                  75                  80

Cys Lys Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn
                85                  90                  95

Phe Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys
            100                 105                 110

Thr Gly Lys Cys
        115
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<210> 64
<211> 7
<212> PRT
<213> Artificial

<220>
<223> 7 residues of a pelB leader sequence

<400> 64

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Ala Ala Gln Pro Ala Met Ala
1               5
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<210> 65
<211> 121
<212> PRT
<213> Artificial

<220>
<223> 2325p4 protein with pelB leader sequence that is 7 amino acid res
idues long.

<400> 65

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Ala Ala Gln Pro Ala Met Ala Lys Pro Lys Glu Asp Arg Glu Trp Glu
1               5                  10                  15

Lys Phe Lys Thr Lys His Ile Thr Ser Gln Ser Val Ala Asp Phe Asn
```

20 25 30

Cys Asn Arg Thr Met Asn Asp Pro Ala Tyr Thr Pro Asp Gly Gln Cys
35 40 45

Lys Pro Ile Asn Thr Phe Ile His Ser Thr Thr Gly Pro Val Lys Glu
50 55 60

Ile Cys Arg Arg Ala Thr Gly Arg Val Asn Lys Ser Ser Thr Gln Gln
65 70 75 80

Phe Thr Leu Thr Thr Cys Lys Asn Pro Ile Arg Cys Lys Tyr Ser Gln
85 90 95

Ser Asn Thr Thr Asn Phe Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro
100 105 110

Val His Phe Val Lys Thr Gly Lys Cys
115 120

<210> 66
<211> 22
<212> PRT
<213> Artificial

<220>
<223> 22 residues of a pelB leader sequence

<400> 66

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
1 5 10 15

Ala Gln Pro Ala Met Ala
20

<210> 67
<211> 136
<212> PRT
<213> Artificial

<220>
<223> Recombinantly produced 2325p4 protein with pelB leader sequence t
hat is 22 amino acid residues long.

<400> 67

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
1 5 10 15

Ala Gln Pro Ala Met Ala Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys
20 25 30

```
Phe Lys Thr Lys His Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys
        35                  40                  45

Asn Arg Thr Met Asn Asp Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys
    50                  55                  60

Pro Ile Asn Thr Phe Ile His Ser Thr Thr Gly Pro Val Lys Glu Ile
65                  70                  75                  80

Cys Arg Arg Ala Thr Gly Arg Val Asn Lys Ser Ser Thr Gln Gln Phe
                85                  90                  95

Thr Leu Thr Thr Cys Lys Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser
            100                 105                 110

Asn Thr Thr Asn Phe Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val
        115                 120                 125

His Phe Val Lys Thr Gly Lys Cys
    130                 135
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<210> 68
<211> 115
<212> PRT
<213> Artificial

<220>
<223> Recombinantly produced 2325p4a protein with methionine in -1 position.

<400> 68

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Met Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His
1               5                   10                  15

Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn
            20                  25                  30

Asp Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Val Asn Thr Phe
        35                  40                  45

Ile His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr
    50                  55                  60

Gly Arg Val Asn Lys Ser Ser Thr Gln Gln Phe Thr Leu Thr Thr Cys
65                  70                  75                  80

Lys Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe
                85                  90                  95
```

Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr
100 105 110

Gly Lys Cys
115

<210> 69
<211> 115
<212> PRT
<213> Artificial

<220>
<223> "Cysteinized" 2325p4-Cys71 protein with methionine in -1 position

<400> 69

Met Lys Pro Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His
1 5 10 15

Ile Thr Ser Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn
20 25 30

Asp Pro Ala Tyr Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe
35 40 45

Ile His Ser Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr
50 55 60

Gly Arg Val Asn Lys Ser Ser Cys Gln Gln Phe Thr Leu Thr Thr Cys
65 70 75 80

Lys Asn Pro Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe
85 90 95

Ile Cys Ile Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr
100 105 110

Gly Lys Cys
115

<210> 70
<211> 176
<212> PRT
<213> Artificial

<220>
<223> "Cysteinized" fusion protein.

<400> 70

Asn Ser Asp Ser Glu Cys Pro Leu Ser His Asp Gly Tyr Cys Leu His

1 5 10 15

Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn
20 25 30

Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys
35 40 45

Trp Trp Glu Leu Arg Gly Gly Ser Gly Gly Pro Gly Gly Ser Lys Pro
50 55 60

Lys Glu Asp Arg Glu Trp Glu Lys Phe Lys Thr Lys His Ile Thr Ser
65 70 75 80

Gln Ser Val Ala Asp Phe Asn Cys Asn Arg Thr Met Asn Asp Pro Ala
85 90 95

Tyr Thr Pro Asp Gly Gln Cys Lys Pro Ile Asn Thr Phe Ile His Ser
100 105 110

Thr Thr Gly Pro Val Lys Glu Ile Cys Arg Arg Ala Thr Gly Arg Val
115 120 125

Asn Lys Ser Ser Cys Gln Gln Phe Thr Leu Thr Thr Cys Lys Asn Pro
130 135 140

Ile Arg Cys Lys Tyr Ser Gln Ser Asn Thr Thr Asn Phe Ile Cys Ile
145 150 155 160

Thr Cys Arg Asp Asn Tyr Pro Val His Phe Val Lys Thr Gly Lys Cys
165 170 175

<210> 71
<211> 528
<212> DNA
<213> Artificial

<220>
<223> DNA of "cysteinized" hEGF fusion protein.

<400> 71
aactctgact ctgaatgccc gctgtctcat gacggttact gcctgcatga cggtgtttgc 60

atgtacatcg aagctctgga caaatacgct tgcaactgcg ttgttggtta catcggtgaa 120

cgttgccagt accgtgacct gaaatggtgg gaactgcgtg gtggttctgg tggtccgggt 180

ggttctaaac cgaaagaaga ccgtgaatgg gaaaaattca aaactaaaca tatcacttct 240

cagtctgttg ctgacttcaa ctgcaaccgt actatgaacg acccggctta cactccggac 300

ggtcagtgca aaccgatcaa cactttcatc cattctacta ctggtccggt taaagaaatc 360

tgccgtcgtg ctactggtcg tgttaacaaa tcttcttgcc agcagttcac tctgactact 420

tgcaaaaacc cgatccgttg caaatactct cagtctaaca ctactaactt catctgcatc 480

acttgccgtg acaactaccc ggttcatttc gttaaaactg gtaaatgc 528

<210> 72
<211> 55
<212> DNA
<213> Artificial

<220>
<223> pET-22b-EGF forward primer

<400> 72
ccaactctga ctctgaatgc ccgctgtctc atgacggtta ctgcctgcat gacgg 55

<210> 73
<211> 54
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:73 EGF forward primer

<400> 73
ggtggttctg gtggtccggg tggttctaaa ccgaaagaag accgtgaatg ggaa 54

<210> 74
<211> 54
<212> DNA
<213> Artificial

<220>
<223> SEQ ID NO:74 EGF reverse primer

<400> 74
agaaccaccc ggaccaccag aaccaccacg cagttcccac catttcaggt cacg 54